Extension, Locomotion, and Proliferation of Osteoblast-like Cells on Metals

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Introduction: To the metallic biomaterials, corrosion resistance and bone tissue compatibility are important to determine the performance of them used for the dental and medical applications. Although titanium (Ti), zirconium (Zr), and gold (Au) show high corrosion resistance, Ti shows the best hard tissue compatibility among them. On the other hand, Ti and Zr are covered by surface passive oxide films, while Au is not. Thus, the deep investigation of this difference in surface properties among these metals makes it possible to elucidate the mechanism of bone formation on materials. Besides, our previous studying [1] show that the sputter deposition of metal on a transparent substrate, such as cover glass, is effective to prepare a cleaner surface than traditional method, such as polishing. In this study, the sputter deposition technique was used to prepare ultra-fine metallic surfaces. And then, a mouse osteoblast-like cell (MC3T3-E1) was employed to have an *in vitro* test. In the initial stage of cell-metal interactions, cellular extension, locomotion, and proliferation behaviors on the different metallic surfaces were studied through the morphological diagnosis detected by fluorescence microscopes. Our study is important for understanding of the cell-biomaterials interactions at cell-metal interface.

Methods: Ti, Zr and Au specimens were prepared by sputter-deposition onto cover glasses. All specimens were sterilized by 70% ethanol before using. A mouse osteoblast-like cell line (MC3T3-E1, RIKEN cell bank, Japan) were cultured in a culture medium. The immunostaining images were observed under a fluorescence microscope (IX71, Olympus, Japan). After 3, 6, 24, 72, and 120 h incubation, the cells were washed with PBS and fixed with 4% paraformaldehyde for 15 min. Then, fixed cells were permeabilized in 1% Triton X-100 in PBS for 5 min, and then primary antibody (mouse anti-vinculin) were added. After overnight incubation, secondary antibodies were added for 1 h. Then cellular F-actin and nuclei were stained by 100 nM rhodamine-phalloidin, and 100 nM Hoechst33342, respectively. Moreover, the cellular proliferation curve was detected by cell number on the metals with a time series analysis using WST-8 Cell Counting Kit (Dojindo, Japan). As a control, cells were incubated on tissue culture treated plastic dishes (TCPS)

Results: The cell number counting results showed that at the first several hours, the cell number collected from TCPS was higher than metallic surface. From 6-hour incubation to 5-day incubation, there was no significant difference of the attached cell number among the samples. Thus, a similar proliferation rate among the Ti, Zr, Au, and also control was obtained. This was also confirmed by the images obtained by optical microscope. Although the adhesion and proliferation behavior of MC3T3-E1 on metals, the different extension and locomotion responses to the metallic surfaces presented by immunostaining images. Figure 1 shows fluorescence images of MC3T3-E1 cells on the metallic surfaces after 3-hour incubation. The upper figures (**Fig. 1a**, **b** and **c**) show the staining of cellular F-acting, which is essential for important cellular functions as the mobility. The shape of cells on Ti (**Fig. 1a**) and Zr (**Fig. 1b**) is a little smaller than the cells on Au (**Fig. 1c**). This indicated that the extension of MC3T3-E1 on Au is better than the cells on Ti and Zr. On the other hand, Lower figures (**Fig. 1d**, **e** and **f**) show the staining of cellular adhesion plaques on the metals, which is labeled with vinculin, a cytoskeletal protein associated with cell-matrix and cell-cell junctions. The clear punctate shape adhesion plaques can be found in side of MC3T3-E1 on Ti (**Fig. 1d**) and Zr (**Fig. 1e**) surfaces. However, the shape of adhesion plaques of cells on Au (**Fig. 1f**) surface became thin fibrous shape and also spread over the outermost of cells. This phenomenon indicates that after 3-hour incubation, the cells on Ti and Zr tend to migration, while, a settlement tend of cells can be obtained. After 24-hour incubation, the shape of adhesion plaques was a thin fibrous shape. In addition, with a long-term incubation, the punctate shape adhesion plaques can be observed again, which may indicate the locomotion of proliferous MC3T3-E1.

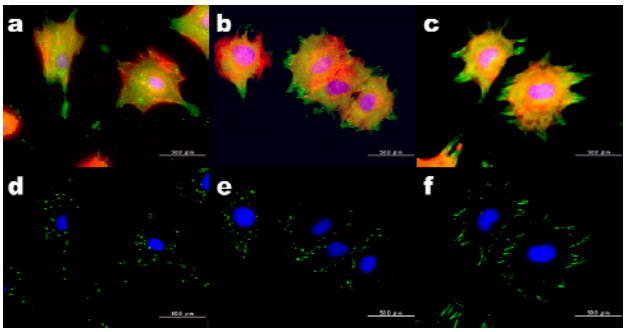

Fig. 1 Fluorescence images of MC3T3-E1 cells on the metallic surfaces (Ti (a,d), Zr (b, e), and Au (c. f)) after 3 h incubation. Staining of adhesion plaques (labeled with vinculin antibodies and with (a, b, and c) or without (d, e, and f) F-actin (labeled with rhodamine-phalloidin). (Nuclei stained with Hoechst 33342, scale bar=50 μm).

Conclusions: In initial stage of cell-metal interactions, similar cellular adhesion and proliferation rate of MC3T3-E1 cells on metals were obtained. However, the extension and locomotion behaviors were different. This work may contribute to understand of the cell-biomaterial interaction at cell-metal interface.

References:

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